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The (+)-isomers of mefloquine and its threo analog are 1.69 to 1.95 times more active than the (-)-isomers against chloroquine-sensitive and chloroquine-resistant Plasmodium falciparum in vitro. This large a differential between the activity of (+)- and (-)-isomers was not observed for other synthetic amino alcohol antimalarial agents containing a piperidine ring. The enantiomers of amino alcohol antimalarial agents in which the amine is part of an acrylic group, such as in halofantrine, displayed little, if any, differential antimalarial activity. Thus, the effect of absolute stereochemistry of the amino alcohol antimalarial agents on antimalarial activity appears to depend upon both the flexibility of the amine portion of the molecule and the structure of the aromatic portion of the molecule.

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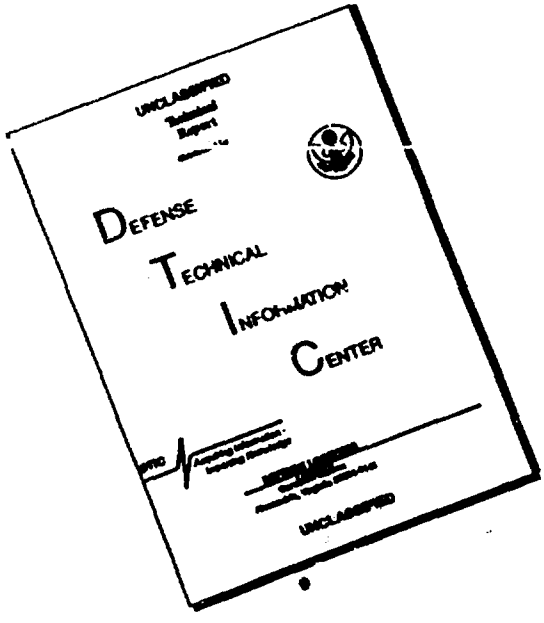

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Plasmodium falciparum: Role of Absolute Stereochemistry in the Antimalarial Activity of Synthetic Amino Alcohol Antimalarial Agents

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KARLE, J. M., OLMEDA, R., GERENA, L., AND MILHOUS, W. K. 1993. *Plasmodium falciparum:* Role of absolute stereochemistry in the antimalarial activity of synthetic amino alcohol antimalarial agents. *Experimental Parasitology* 76, 345–351. The (+)-isomers of mefloquine and its *threo* analog are 1.69 to 1.95 times more active than the (−)-isomers against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum in vitro*. This large a differential between the activity of (+)- and (−)-isomers was not observed for other synthetic amino alcohol antimalarial agents containing a piperidine ring. The enantiomers of amino alcohol antimalarial agents in which the amine is part of an acyclic group, such as in halofantrine, displayed little, if any, differential antimalarial activity. Thus, the effect of absolute stereochemistry of the amino alcohol antimalarial agents on antimalarial activity appears to depend upon both the flexibility of the amine portion of the molecule and the structure of the aromatic portion of the molecule.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Plasmodium falciparum;* Protozoa, parasitic; Malaria; Enantiomers; Mefloquine; Halofantrine; Enpiroline; High-performance liquid chromatography (HPLC); *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes).

INTRODUCTION

An understanding of the three-dimensional structural components responsible for antimalarial activity should aid the design of new drugs to treat the increasingly abundant resistant strains of *Plasmodium falciparum*. The naturally occurring cinchona alkaloids show activity differences dependent upon the absolute stereochemistry of their amine and hydroxyl groups. *In vitro* studies (Karle *et al.* 1992) with the cinchona alkaloids have demonstrated that quinidine (the 8*R*,9*S* alkaloid) (Fig. 1) is 2.3 and 2.8 times more active than quinine (the 8*S*,9*R* alkaloid) against chloroquine-sensitive Sierra Leone D-6 and chloroquine-resistant Indochina W-2 *P. falciparum*, respectively. Likewise, dihydroquinidine was approximately twice as active as dihydroquinine against both the chloroquine-sensitive and chloroquine-resistant clones. These results are consistent with the findings of Wesche and Black (1990) who found quinidine to be 2.5 times more active than quinine and found cinchonine (the demethoxyl analog of quinidine) to be 2.8 times more active than cinchonidine (the demethoxyl analog of quinine) *in vitro* against Papua New Guinea FCQ-27/PNG *P. falciparum*. Subsequently, Basco *et al.* (1992) reported that quinidine was 2.2 and 3.2 times more active than quinine *in vitro* against Cameroon chloroquine-resistant FCM 29 and Ivory Coast chloroquine-sensitive L-3 *P. falciparum* strains, respectively. In addition, quinidine was twice as effective as quinine against induced McClendon *P. falciparum* clinical infections (Taggart *et al.* 1948) and was more potent clinically than quinine against Thai *P. falciparum* (White *et al.* 1981; Phillips *et al.* 1985).

In this study, the relative potency of the (+)- and (−)-isomers of synthetic amino alcohol antimalarial agents was quantitated to determine if absolute stereochemistry plays a role in efficacy of the synthetic agents as it does for the naturally occurring cinchona alkaloids. We also wished to de-

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termine if the (+)- and (−)-isomers of synthetic amino alcohol antimalarial agents possess the same relative antimalarial activity to both chloroquine-sensitive and chloroquine-resistant malaria.

Material and Methods

Antimalarial agents. Racemic enpiroline phosphate, halofantrine hydrochloride, mefloquine hydrochloride, WR 30,090 hydrochloride, WR 33,063 hydrochloride, WR 122,455 hydrochloride, WR 165,355 hydrochloride, and WR 177,602 hydrochloride were supplied by the Division of Experimental Therapeutics Chemical Repository. Enpiroline was resolved by Ash Stevens, Inc. (Detroit, MI), and all of the other racemates were resolved by Research Triangle Institute (Research Triangle Park, NC). All of the resolved antimalarial agents were in the same salt form as the corresponding racemate. The enantiomeric purity of the resolved antimalarial agents was established by using HPLC and ultraviolet absorbance at 254 nm. The HPLC columns used were the Daicel Chiralpak AD, Chiralcel OD, and Chiralcel OG columns, 4.6 × 250 mm, 10-μm particle size (J. T. Baker, Inc., Phillipsburg, NJ) and the Pirkle Covalent L-leucine column, 10 × 250 mm, 5-μm particle size (REGIS, Morton Grove, IL). The eluant was 2-propanol/hexane/0.4% diethylamine. Peak areas were quantitated using the Axxiom Chromatography Data System (Calabasas, CA).

Parasites and culture conditions. Two *P. falciparum* clones designated Indochina (W-2) and Sierra Leone (D-6) were maintained in a 5-ml suspension of human type A+ erythrocytes (6% hematocrit) containing less than 2% parasitized cells in RPMI 1640 culture medium (GIBCO, Grand Island, NY) with 32 m*M* $NaHCO_3$, 25 m*M* Hepes, and 10% (v/v) heat-inactivated human plasma at 37°C in sealed 50-ml culture flasks under a 5% O_2/5% CO_2/90% N_2 atmosphere. The clones were derived by single erythrocyte micromanipulation (Oduola *et al.* 1988) from patient isolates obtained from the Centers for Disease Control (Atlanta, GA) in 1980 and 1982, respectively, and represent infections acquired in Vietnam or Sierra Leone. The Indochina clone is resistant to the antimalarials chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone clone is resistant to mefloquine, but sensitive to chloroquine, quinine, sulfadoxine, and pyrimethamine (Milhous *et al.* 1989).

Drug susceptibility testing. The assays were conducted *in vitro* using a modification of the semiautomated microdilution technique of Desjardins *et al.* (1979) and Milhous *et al.* (1985). The enantiomers or racemic compounds were dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium with 10% heat-inactivated human plasma. The test compounds were subsequently further diluted into microtiter wells using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of 0.032 to 500 ng/ml or 0.0032 to 50 ng/ml. Each dilution was assayed in duplicate. The test compounds were incubated with parasite inocula (0.5% parasitemia and a 1% hematocrit) for 24 hr at 37°C prior to the addition of 0.37 μCi of [G-^{3}H]hypoxanthine monochloride (17.2 Ci/mmol, NEN Research Products, Dupont Co., Boston, MA). After further incubation for 18 hr at 37°C, particulate matter was harvested from each microtiter well onto filter paper mats using an automated cell harvester (MACH II, TOM-TEC, Orange, CT). Dried mats were counted in a scintillation spectrometer (Model LKB 1205 Betaplate, Wallac, Gaithersburg, MD). The IC_{50} value, k value, for each compound was calculated by fitting the concentration–response data to the IHILL equation:

$$y = \frac{\max}{\left(1 + \left(\frac{x}{k}\right)^{n}\right)},$$

where x is the concentration of the test compound, y is the amount of incorporated tritium, and max, k, and n are the constants whose values are estimated by nonlinear regression.

Statistical analysis. The two-tailed paired t test was applied to the IC_{50} values of pairs of (+)- and (−)-enantiomers assayed simultaneously.

Results

Enantiomeric purity of the resolved antimalarial agents. The enantiomeric purity of the test compounds (Fig. 1) was assayed by HPLC and ranged from 91.6 to over 99.5% (Table I). The (−)-isomers eluted prior to the (+)-isomers from the Chiralpak AD, Chiralcel OD, and Chiralcel OG columns whereas (+)-halofantrine eluted prior to (−)-halofantrine from the L-leucine column.

Antimalarial activity. The synthetic amino alcohol antimalarial agents listed in Table II are 5.2 to 26.5 times more active against the chloroquine-resistant Indochina W-2 clone than they are against the chloroquine-sensitive Sierra Leone D-6 clone. This is true whether or not the amine is part of an acyclic group or is part of a piperidine ring in the *erythro* or *threo* configuration. This is in contrast to the cinchona alkaloids (Karle *et al.* 1992) where the amine group is

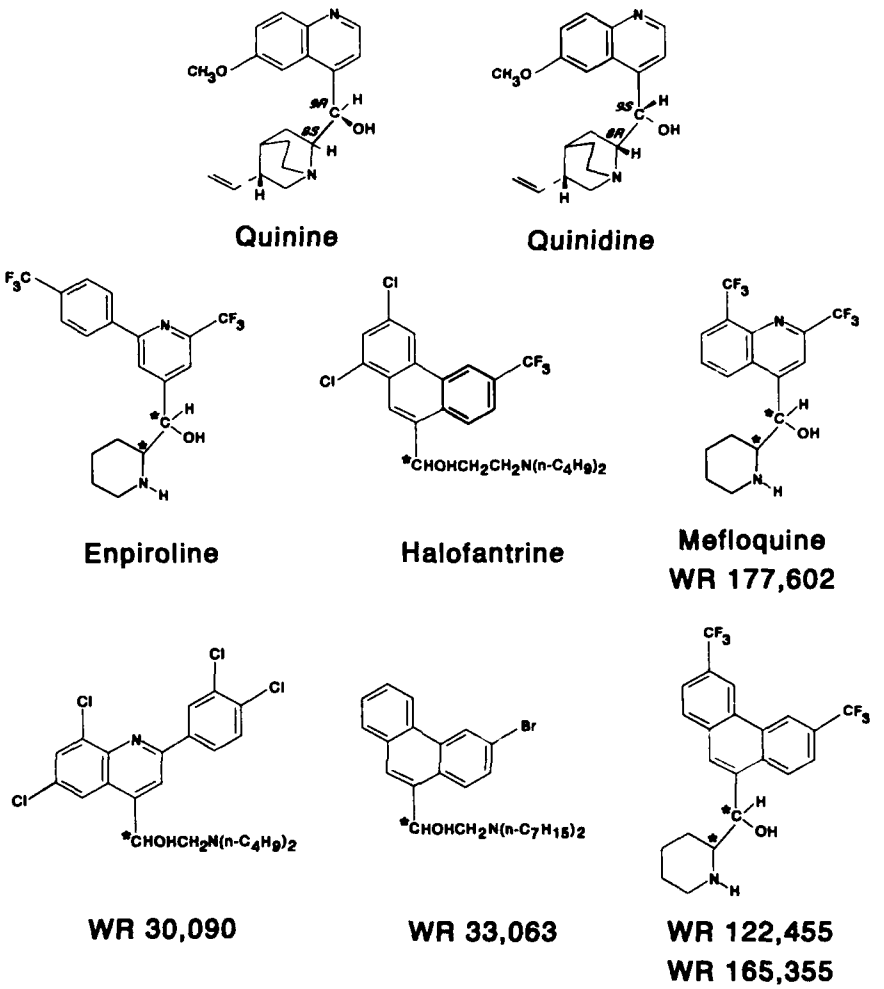

FIG. 1. Chemical structures. The asterisks indicate asymmetric carbon atoms. Dihydroquinine and dihydroquinidine result from saturation of the vinyl group in quinine and quinidine, respectively. 9-Epiquinine and 9-epiquinidine are the 8*S*,9*S* and 8*R*,9*R* analogs, respectively, of quinine and quinidine. Mefloquine and WR 177,602 are diastereomers. Racemic *erythro* mefloquine is an equal mixture of the *RS* and *SR* isomers. Racemic *threo* WR 177,602 is an equal mixture of the *RR* and *SS* isomers. Racemic *threo* enpiroline is an equal mixture of the *RR* and *SS* isomers. From nuclear magnetic resonance studies, WR 122,455 appears to be the *erythro* diastereomer, and WR 165,355 appears to be the *threo* diastereomer (Olsen 1972; Carroll and Blackwell 1974).

part of a bulkier bicyclo ring system and where the *erythro* quinine, quinidine, dihydroquinine, and dihydroquinidine are all at least 3.3 times less active against the W-2 clone than the D-6 clone. However, like the synthetic antimalarial agents, the *threo* 9-epiquinine and 9-epiquinidine are 2.6 to 2.9 times more active against the W-2 clone than the D-6 clone (Karle *et al.* 1992).

Of all the synthetic amino alcohol antimalarial agents listed in Table II, only the enantiomers of the *erythro* mefloquine and its *threo* analog WR 177,602 demonstrated differential IC_{50} activities within the same *P. falciparum* clone approaching the 2.3- to 2.9-fold difference observed between quinidine versus quinine and dihydroquinidine versus dihydroquinine (Karle *et al.* 1992).

TABLE I
Enantiomeric Purity of the Resolved Amino Alcohol Antimalarial Agents as Determined by HPLC

Compound	Enantiomeric purity (%)[a]	HPLC column	Percentage 2-propanol	Retention time (min)	Flow rate (ml/min)	Resolution
(+)-Enpiroline	>99.5	Chiralpak AD	5	12.7	1	Baseline
(−)-Enpiroline	>99.5			9.3		
(+)-Halofantrine	>99.5	L-Leucine	3	10.5	2	Baseline
(−)-Halofantrine	>99.5			11.1		
(+)-Mefloquine	98.9	Chiralpak AD	10	13.7	1	Baseline
(−)-Mefloquine	>99.5			5.3		
(+)-WR 30,090	95.4	Chiralcel OG	1	7.4	1	Baseline
(−)-WR 30,090	93.4			5.9		
(+)-WR 33,063	94.1	Chiralcel OG	0.5	8.6	1	Baseline
(−)-WR 33,063	95.8			6.5		
(+)-WR 122,455	91.6	Chiralcel OG	5	9.5	1	Baseline
(−)-WR 122,455	>99.5			7.6		
(+)-WR 165,355	99.2	Chiralcel OD	2	18.0	1	Baseline
(−)-WR 165,355	98.1			14.5		
(+)-WR 177,602	>99.5	Chiralcel OD	2	19.0	1	$R_S = 1.23$[b]
(−)-WR 177,602	>99.5			16.9		

[a] Percentage of desired isomer in sample.
[b] R_S = the distance between two peak centers divided by the average base peak width.

For both mefloquine and WR 177,602, the (+)-isomer was 1.69 to 1.95 times more active than the (−)-isomer. Although the IC_{50} value varied some from experiment to experiment, each individual experiment consistently showed the (+)-isomer of mefloquine and WR 177,602 to be the more potent isomer as demonstrated in the standard deviation for the ratio of IC_{50} values. The compounds other than mefloquine and WR 177,602 which contain a piperidine ring displayed no more than a 1.30-fold difference in the IC_{50} values of their individual enantiomers within the same *P. falciparum* clone. The acyclic compounds displayed no more than a 1.14-fold difference in the IC_{50} values of their individual enantiomers within the same *P. falciparum* clone. For all of the synthetic amino alcohol antimalarial agents, the ratio of IC_{50} values of the individual enantiomers were nearly identical for both the chloroquine-sensitive clone and the chloroquine-resistant clone.

Discussion

The data in Table II show that both enantiomers of the synthetic amino alcohol antimalarial agents are potent antimalarial agents *in vitro* against both the chloroquine-sensitive and chloroquine-resistant *P. falciparum* clones. This is consistent with the results of Childs *et al.* (1984) who found both enantiomers of WR 122,455 and WR 165,355 to be active *in vitro* against the chloroquine-sensitive Malayan Camp and the chloroquine-resistant Vietnam Smith *P. falciparum* strains and with the results of Basco *et al.* (1992) who found both enantiomers of enpiroline, halofantrine, and mefloquine to be active *in vitro* against the chloroquine-sensitive Ivory Coast L-3 and the Cameroon FCM29 *P. falciparum* strains. *In vivo* data has also shown both enantiomers of *erythro* mefloquine and *threo* WR 177,602 as well as both enantiomers of the diastereomers WR 122,455 and WR 165,355, halofantrine, WR 33,090, and WR 33,093 to be active against *Plasmodium berghei* in mice (Carroll and Blackwell 1974; Carroll *et al.* 1978). Karle and Karle (1989) had predicted that both enantiomers of enpiroline should possess antimalarial activity since one enantiomer of enpiroline superimposes well structurally with quinine

TABLE II
Susceptibility of *Plasmodium falciparum* to the Racemic Amino Alcohol Antimalarial Agents and Their Individual Enantiomers[a]

Compound		D-6 Clone		W-2 Clone	
		IC-50 (n*M*)	Ratio of IC-50 values[b]	IC-50 (n*M*)	Ratio of IC-50 values[b]
WR 177,602	Racemic	16.6 ± 6.8 (5)		1.68 ± 0.64 (5)	
	(+)-Isomer	13.0 ± 6.5 (5)	1.85 ± 0.34 (5)[c]	1.40 ± 0.67 (5)	1.95 ± 0.21 (5)[d]
	(−)-Isomer	22.5 ± 8.6 (5)		2.71 ± 1.21 (5)	
Mefloquine	Racemic	34.4 ± 6.9 (5)		3.87 ± 1.07 (5)	
	(+)-Isomer	23.4 ± 3.8 (5)	1.81 ± 0.17 (5)[e]	4.09 ± 2.21 (5)	1.69 ± 0.16 (5)[c]
	(−)-Isomer	42.3 ± 7.2 (5)		6.61 ± 3.05 (5)	
Enpiroline	Racemic	16.4 ± 4.9 (4)		2.85 ± 1.64 (3)	
	(+)-Isomer	14.4 ± 7.0 (4)	1.15 ± 0.21 (4)	2.23 ± 0.60 (3)	1.22 ± 0.25 (3)
	(−)-Isomer	15.6 ± 5.1 (4)		2.74 ± 1.03 (3)	
WR 165,355	Racemic	5.53 ± 0.21 (2)		0.77 ± 0.54 (2)	
	(+)-Isomer	4.87 ± 0.92 (2)	0.87 ± 0.03 (2)	0.91 ± 0.72 (2)	0.82 ± 0.19 (2)
	(−)-Isomer	4.24 ± 0.94 (2)		0.88 ± 0.75 (2)	
WR 122,455	Racemic	12.3 ± 1.0 (2)		2.37 ± 1.67 (2)	
	(+)-Isomer	12.3 ± 0.7 (2)	1.09 ± 0.05 (2)	1.74 ± 1.24 (2)	1.30 ± 0.10 (2)
	(−)-Isomer	13.3 ± 0.1 (2)		2.12 ± 1.43 (2)	
WR 30,090	Racemic	17.0 ± 3.0 (2)		2.93 ± 2.23 (2)	
	(+)-Isomer	15.5 ± 1.6 (2)	1.10 ± 0.18 (2)	2.30 ± 1.71 (2)	1.13 ± 0.16 (2)
	(−)-Isomer	17.3 ± 4.6 (2)		2.85 ± 2.27 (2)	
WR 33,063	Racemic	81.3 ± 7.1 (2)		3.07 ± 1.76 (2)	
	(+)-Isomer	109 ± 31 (2)	1.02 ± 0.02 (2)	3.74 ± 1.87 (2)	0.88 ± 0.05 (2)
	(−)-Isomer	111 ± 33 (2)		3.15 ± 1.42 (2)	
Halofantrine	Racemic	5.57 ± 0.69 (2)		0.56 ± 0.08 (2)	
	(+)-Isomer	6.03 ± 0.72 (2)	0.95 ± 0.05 (2)	0.58 ± 0.11 (2)	1.03 ± 0.02 (2)
	(−)-Isomer	5.72 ± 0.40 (2)		0.60 ± 0.12 (2)	

[a] Mean ± SD, number of determinations in parentheses.
[b] Ratio of the IC-50 value of the (−)-isomer to the IC-50 value of the (+)-isomer.
[c] $P < 0.01$.
[d] $P = 0.01$.
[e] $P = 0.001$.

and the other enantiomer superimposes well structurally with quinidine. The results obtained by Basco *et al.* (1992) and obtained in this study confirm this prediction.

The more rigid the molecule, the more likely an enantiomeric difference in potency will be observed against *P. falciparum*. As discussed in the introduction, quinidine, with its bicyclo ring structure (Fig. 1), is two to three times more active than quinine *in vitro* against both African and Asian *P. falciparum*. When the bicyclo ring structure is simplified to a piperidine ring, the results are variable. The (+)-isomers of mefloquine and WR 177,602 were more active than the (−)-isomers against the Sierra Leone and the Indochina *P. falciparum* clones (Table II), but the enantiomers of mefloquine were equally active against the Ivory Coast and Cameroon *P. falciparum* strains (Basco *et al.* 1992). The enantiomers of enpiroline were essentially equally active against both the Asian and African *P. falciparum* (Table II and Basco *et al.* 1992). The enantiomers of WR 122,455 and WR 165,355 were almost equally active against the *P. falciparum* clones used in this study (Table II) and in Childs *et al.* (1984). Finally, none of the acyclic compounds in this study nor in the

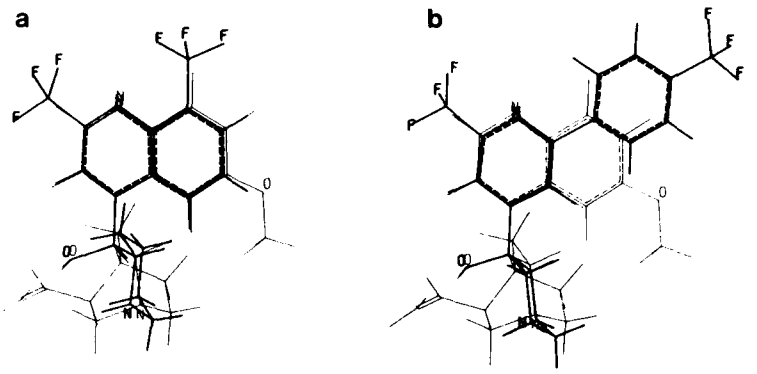

FIG. 2. Superposition of (a) quinidine and one enantiomer of mefloquine and (b) quinidine and one enantiomer of enpiroline. Quinidine was drawn with narrow lines. Mefloquine and enpiroline were drawn with thick lines. All of the heteroatoms are labeled. The conformation of the molecules was determined by X-ray crystallography (Karle and Karle 1989, 1991) with the exception of the piperidine ring of enpiroline which has been rotated such that its amine group superimposes with the amine group of quinidine.

Basco *et al.* study (1992) displayed any significant differential activity by the enantiomers.

The structure of the aromatic ring system is important to the differential activity exhibited by the antimalarial agents containing a piperidine ring. Like quinine and quinidine, both mefloquine and WR 177,602 contain a quinoline ring (Figs. 1 and 2a) and both exhibit differential activity by their enantiomers. However, when the aromatic ring system is altered to a biphenyl analog in enpiroline and to a phenanthrene in WR 122,455 and WR 165,355, none of these compounds display a significant enantiomeric difference in antimalarial activity. Molecular graphics illustrates the additional bulk of the benzene ring in enpiroline compared to a quinoline ring (Fig. 2b), a possible reason for the lack of differential antimalarial activity. A phenanthrene ring also adds bulk in the same region.

In summary, mefloquine and its *threo* isomer WR 177,602 show up to a 1.95-fold difference in potency of their respective (+)- and (−)-isomers *in vitro* which approaches the two- to threefold difference observed for quinine and quinidine. None of the enantiomers of acyclic analogs nor other compounds containing a piperidine ring displayed significant differences in the potency of their respective enantiomers. The relative potency (the ratio of IC_{50} values) of the enantiomers of the synthetic amino alcohol antimalarial agents was essentially the same for the chloroquine-sensitive and the chloroquine-resistant *P. falciparum*.

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